REMARK

Applicant intends this response to be a complete response to the Examiner's **16 January 2007** Non-Final Office Action. Applicant has labeled the paragraphs in his response to correspond to the paragraph labeling in the Office Action for the convenience of the Examiner.

DETAILED ACTION

Claims 1-27 are pending. Claims 1-8, 14-20, 25 and 26 are withdrawn from consideration. Claims 9-13, 21-24 and 27 are examined.

Election/Restrictions

Applicant's election with traverse of Group IV, claims 9-13, 21-24 and 27, and in particular, now drawn to the mutation Trp278X, in the reply filed on 7/6/2006, is acknowledged.

The Examiner Contends as follows:

> The traversal is on the ground(s) that that all recited mutations are, mutations of the AIPL1 encoding or regulatory sequence, and the databases are such that the search should not represent a burden as the searches are now highly automated. Also the mutations are specific and represent a location of sites in the AIPLI encoding or regulatory sequence that represent potentially signatures of a retinal disease, which can occur individually or collectively. Thus, applicant argues that a search for one mutation may well additionally identify other members of the same group. Applicant request that if a generic claim is deemed patentable for the Trp278X mutant, then the examiner search and examine all other non-elected inventions, as required by the MPEP.
>
> This is not found wholly persuasive because the different mutations represent materially different nucleic acid/protein sequence variants of the AIPII gene/protein. Applicant does not argue that these sequences are obvious over one another.
>
> The Requirement for Restriction/Election, mailed 4/12/2006, stated that the mutants selected from the group consisting of Ala336.DELTA.2, Trp278X, Cys239Arg, M79T, L88X, V961, T1241, P376S, Q163X, A197P, IVS2-2, G262S, R302L, P351 D12, Cys42X (TGT.fwdarw.TGA), Val33ins 8 bp (GTGATCTT), Leu257del 9 bp (CTCCGGCAC), where considered to be each distinct inventions, as they represented materially different sequence of nucleotides and amino acids. Thus the said Requirement did not consider these mutants to be species but rather considered them to be distinct inventions.
>
> The art and specification indicates that these mutations can be associated with different families, and so have apparently etiology and pathogenesis. Thus these mutation Inventions appear to be distinct, in that they have materially different modes of operation, function and effect.
>
> Furthermore, if these mutations are claimed as sequences subject to the nucleotide/amino acid sequence rules, then as directed in MPEP 803.04, examination is limited to a reasonable number, which is generally considered to be less than ten. The number of mutations claimed number more than ten.

Finally, the examiner respectfully finds that the elected mutation of Trp278X, is anticipated in the prior art. See below rejections.

The examiner is willing to consider more particular reasons as to why the mutations would not constitute an undue administrative burden, in view of these specific considerations.

Applicant acknowledge the Examiner's stance on the election.

3. **Claims 1-8, 14-20, 25 and 26** stand withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected Invention, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on 7/6/2006.

Applicants acknowledge the withdrawal of these claims from consideration and expressly reserves the right to file continuations or divisionals to address unelected subject matter.

Priority

This application was filed 1/17/2001, which is the effective filing date.

Applicants disagree with the effective filing date as this application claim priority of United State Patent Application Serial No. 60/331362 filed January 4, 2001 and sent bearing Express Mail Label EL 389 348 319 US to the United States Patent and Trademark Office on January 4, 2001. This application was originally assigned non-provisional serial no. 098/754842, but was converted to a provisional action by a June 22, 2001 petition to convert that was granted on March 27, 2002 assigning provisional number 60/331362.

Information Disclosure Statement

The information disclosure statement filed 11/05/2006 fails to comply with the provisions of 37 CFR 1.97, 1.98 and MPEP § 609 because the citation to Hameed et al. (3/2000) and to Sohocki et al (1/2000) journal source information. The IDS has been placed in the application file, but the information referred to therein in regard to said references of Hameed et al. (3/2000) and Sohocki et al (1/2000) have not been considered as to the merits. Applicant is advised that the date of any re-submission of any item of information contained in this information disclosure statement or the submission of any missing element(s) will be the date of submission for purposes of determining compliance with the requirements based on the time of filing the statement, including all certification requirements for statements under 37 CFR 1.97(e). See MPEP § 609.05(a).

Oath/Declaration

6. The oath or declaration is defective. A new oath or declaration in compliance with 37 CFR 1.67(a) identifying this application by application number and filing date is required. See MPEP §§ 602.01 and 602.02.

The oath or declaration is defective because:

Non-initialed and/or non-dated alterations have been made to the oath or declaration. See 37 CFR 1.52(c).

Applicants will submit a new Oath/Declaration shortly.

Specification

7. Applicants disclose nucleotide sequences in the drawings, particularly Figures 1 and 9, that must be identified by a SEQ ID number, pursuant to 37 CFR 1.821(d), which states: "Where the description or claims of a patent application discuss a sequence listing that is set forth in the 'Sequence Listing' in accordance with paragraph (c) of this section, reference must be made to the sequence by use of the assigned identifier, in the text of the description or claims, even if the sequence is also embedded in the text of the description or claims of the patent application." The identification of the sequences by SEQ ID numbers may be in the Brief Description of the Figures; or in the drawings themselves.

Applicants are having new drawings with SEQ. ID prepared and will submit them in a supplemental response.

Claim Rejections - 35 USC § 112

9. **Claims 9-13 and 27** stand rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement.

The Examiner Contends as follows:

> The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This rejection is for lack of written description.
>
> Claim 9-13 are drawn to a method to determine if an animal has a retinal disease or has a propensity to pass a retinal disease to offspring, comprising the steps of: (A) extracting polynucleotide from a cell or sample; (B) determining if the polynucleotide contains a mutation in an AIPLI encoding or regulating region; and (C) correlating the presence of the mutation as an indication of a retinal disease or a propensity to pass a retinal. disease to offspring.

Claim 27 is drawn to a method to determine if a cell or sample has an APL1 mutation comprising: (A) extracting polynucleotide from a cell; (B) amplifying polynucleotides which encode APL1; and (C) determining if the polynucleotide contains a Trp278X mutation; (D) correlating the presence of the mutation as an indication of a retinal disease or a propensity to pass a retinal disease to offspring.

The instant specification, at p. 13, lines 13-18, discloses retinal diseases and disorders as including Leber's congenital amaurosis, juvenile retinitis pigmentosa (RP), dominant cone-rod dystrophy, and other inherited and/or acquired retinopathies. The instant specification, at p. 13, lines 13-18, states that the present invention can be used as a diagnosis and/or treatment of inherited and/or acquired retinopathies in animals including humans and/or development of animal models for diseases caused by or related to mutation in AIPL1. Thus the claim term retinal diseases broadly encompasses inherited and acquired retinopathies.

Vas-Cath Inc. v. Mahurkar, 19USPQ2d 1111, clearly states that "applicant must convey with reasonable clarity to those skilled in. the art that, as of the filing date sought, he or she was in possession of the invention. The invention is, for purposes of the `written description' inquiry, whatever is now claimed." (See page 1117.) The specification does not "clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed." (See Vas-Cath at page 1116).

One of skill in the art cannot envision the genus of retinal diseases that may be determined by detecting a mutation to an AIPLI gene. The specification provides that Leber's congenital amaurosis may be so detected, but the instant specification does not provide the mutant genes or proteins that are capable of inducing any retinal disease, other than Leber's congenital amaurosis. Adequate written description requires more than a mere statement that it is part of the invention and reference to a potential method of isolating it. The mutant genes are required. See Fiers v. Revel, 25 USPQ2d 1601 at 1606 (CAFC 1993) and Amgen Inc. v. Chugai Pharmaceutical Co. Ltd., 18 USPQ2d 1016.

One cannot describe what one has not conceived. See Fiddes v. Baird, 30 USPQ2d 1481 at 1483. In Fiddes, claims directed to mammalian FGF's were found to be unpatentable due to lack of written description for that broad class. The specification provided only the bovine sequence.

Therefore, only Leber's congenital amaurosis, but not the full breadth of the claims encompassing any retinal disease, meets the written description provision of 35 U.S.C. §112, first paragraph. Applicant is reminded that Vas-Cath makes clear that the written description provision of 35 U.S.C. §112 is severable from its enablement provision (see page 1115).

Applicants have amended the claims to Leber's congenital amaurosis, but reserves the right to file claims to other retinal diseases in a continuation application.

11. **Claims 9-13, 21-24 and 27** stand rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Examiner Contends as follows:

Claim 9 recites the limitation "a retinal disease" in lines 2 and 7. There is uncertain antecedent basis for this limitation in the claim because it is not clear if this is the same as "a retinal disease", in line 1.

Claim 21 recites the limitation "in step (b)" in line 4-5 and "step (e)" in line 11. There is insufficient antecedent basis for these limitations in the claim.

Claim 22 recites the limitation "a patient" in 1. There is uncertain antecedent basis for this limitation in the claim because it is not clear if this patient describes the same patient of "a patient sample", in claim 21, lines 1-2.

Claim 27 recites the limitation "a cell" in line 3. There is uncertain antecedent basis for this limitation in the claim because it is not clear if this is the same as "a cell", in line 1.

Applicants

Claim Rejections - 35 USC § 102

13. Claims 9-13, 21-24 and 27 are rejected under 35 U.S.C. 102(b) as being anticipated by Sohocki et al., Nature Genetics, Jan. 1, 2000, Vol. 24, pp. 79-83.

The Examiner Contends as follows:

The claims, (as in claim 9 et seq.), are drawn to a method to determine if an animal has a retinal disease or has a propensity to pass a retinal disease to offspring, comprising the steps of: (A) extracting polynucleotide from a cell or sample; (B) determining if the polynucleotide contains a mutation in an AIPLI encoding or regulating region; and (C) correlating the presence of the mutation as an indication of a retinal disease or a propensity to pass a retinal disease to offspring; and variations thereof.

Also, the claims, (as in claim 21 et seq.), are drawn to methods for determining the presence of an APLI mutant in a patient sample, which comprises: (A) isolating polynucleotide extracted from the patient sample; (B) hybridizing a detectably labeled oligonucleotide to the polynucleotide isolated in step (b), the oligonucleotide having at its 3' end at least 15 nucleotides complementary to a wild type polynucleotide sequence having at least one mutation; (C) attempting to extend the oligonucleotide at its 3'-end; (D) ascertaining the presence or absence of a detectably labeled extended oligonucleotide; and (E) correlating the presence or absence of a detectably labeled extended oligonucleotide in step (e) with the presence or absence of a AIPLI Trp278X mutation; and variations thereof.

Also, the claims, (as in claim 27), are drawn to a method to determine if a cell or sample has an APL1 mutation comprising: (A) extracting polynucleotide from a cell; (B) amplifying polynucleotides which encode APL1; and (C) determining if the polynucleotide contains a Trp278X mutation; (D) correlating the presence of the mutation as an indication of a retinal disease or a propensity to pass a retinal disease to offspring; and variations thereof.

Sohocki et al., Nature Genetics, Jan. 1, 2000, Vol. 24, pp. 79-83, throughout the publication, and abstract disclose methods to determine if an animal has a retinal

disease or has a propensity to pass a retinal disease to offspring, (see, e.g., Fig. 5), comprising the steps of: (A) extracting polynucleotide from a cell or sample, (e.g., p. 81, para 1); (B) determining if the polynucleotide contains a mutation in an AIPLI encoding or regulating region, (see e.g., Fig. 2, demonstrating mutant sequences, and p. 80, teaching elected mutation Trp278X); and (C) correlating the presence of the mutation as an indication of a retinal disease or a propensity to pass a retinal disease to offspring, (see, e.g., Fig. 5); and as in instant claims 9-13.

Sohocki et al., throughout the publication and in the abstract, disclose methods for determining the presence of an APLI mutant in a patient sample, including members of a Pakistani family, LCA4 family, which comprises: (A) isolating polynucleotide extracted from the patient sample; (B) hybridizing a detectably labeled oligonucleotide to the polynucleotide isolated, (see, e.g., Fig. 1), the oligonucleotide having at its 3' end at least 15 nucleotides complementary to a wild type polynucleotide sequence having at least one mutation, (see, e.g., Figure 2); (C) attempting to extend the oligonucleotide at its 3'-end, (see, e.g., Fig. 1, Methods Section, p. 81, para 4-5, p. 82, para 2, p. 83, para 2); (D) ascertaining the presence or absence of a detectably labeled extended oligonucleotide; and (E) correlating the presence or absence of a detectably labeled extended oligonucleotide with the presence or absence of a AIPLI Trp278X mutation . (see p. 80, para 1-6, Fig. 5); as in instant claims 21-24. Sohocki et al., in the abstract, teach taking a patient sample prior to isolation. Sohocki et al., at Fig. 1, and p. 81, para 6, teach amplification, hybridization, and fluorescence in situ hybridization (fluorochrome label), northern blot (radioisotope label), and digoxygenin in situ hybridization (enzyme label); as in instant claims 21-24.

Sohocki et al, throughout the publication, disclose method to determine if a cell or sample has an APL1 mutation comprising: (A) extracting polynucleotide from a cell; **(B)** amplifying polynucleotides which encode APL1; and (C) determining if the polynucleotide contains a Trp278X mutation; (D) correlating the presence of the mutation as an indication of a retinal disease or a propensity to pass a retinal disease to offspring; as in instant claim 27.

Applicants have amended the specification to include priority to provisional 60/331362 filed 01/04/2001 was filed prior to one year before the entire cited reference become publically available. Although the abstract was available prior to 1/4/2000, the abstract did not include the sequence information, which did not become available until some time after 1/4/2000. Therefore, the cited reference is not proper 102(b) reference as it is the inventors work and the a patent application was filed prior to the publication of an enabling disclosure of the subject matter of the current claims. Applicants, therefore, respectfully request withdrawal of this section 102(b) rejection.

14. Claims 9, 12, 13 and 27 are rejected under 35 U.S.C. 102(a) as being anticipated by Damji, et al., American Journal of Human Genetics, Oct. 2000, Vol. 67, No. 4 Supplement 2, pp. 382, Abstract 2142.

The Examiner Contends as follows:

> Damji, et al., throughout the abstract, teach a method to determine if a human patient, (understood here to encompass broadly "an animal", as in the claim) has a retinal disease or has a propensity to pass a retinal disease to offspring, comprising the steps of: (A) extracting polynucleotide from a cell or sample; (B) determining if the polynucleotide contains a mutation in an AIPL1 encoding or regulating region; and (C) correlating the presence of the mutation as an indication of a retinal disease or a propensity to pass a retinal disease to offspring; and wherein the determining is done via sequencing, (as in claim 12); and wherein the mutation is Trp278X, as in claim 13.

Applicants are somewhat mystified by the Damji et al. rejection. Applicants' 2000 publication clearly antedates this reference and as the provisional application to which this application claims priority was filed less than one year after an enabling publication. Applicants therefore, respectfully request withdrawal of this reference as being clearly antedated by Applicants 2000 article.

Having fully responded to the Examiner's Non-Final Office Action, Applicant respectfully urges that is application be passed onto allowance.

The Commissioner is authorized to credit or debit deposit account no. 501518 as needed in filing this response.

If it would be of assistance in resolving any issues in this application, the Examiner is kindly invited to contact applicant's attorney Robert W. Strozier at 713.977.7000

Respectfully Submitted,

Date: **March 12, 2007**

Robert W. Strozier, Reg. No. 34,024

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MAR 12 2007
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UNITED STATES
PATENT AND
TRADEMARK OFFICE

UNDER SECRETARY OF COMMERCE FOR INTELLECTUAL PROPERTY AND
DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE
WASHINGTON, D.C. 20231
www.uspto.gov

March 27, 2002

Robert W. Strozier, PLLC
2925 Briarpark, Suite 930
Houston, TX 77042

In re Application of:
Sohocki, et al.
Application No.: 09/754842
Filed: **January 04, 2001**
Attorney Docket No.: 25630/16UTL

DECISION ON PETITION

This is a decision on your petition received in the Patent and Trademark Office on June 22, 2001, treated as a petition under 37 CFR 1.53(b)(2)(ii) to convert the above identified application to a Provisional application under 35 U.S.C. 111 (b) and 37 CFR 1.53(b)(2).

The petition is granted.

The application will be processed in the Office of Initial Patent Examination (OIPE) as a Provisional application under 35 U.S.C. 111(b) and 37 CFR 1.53(b)(2), including the assignment of a new Provisional application number.

The Provisional application serial number is **60/331362.** The filing receipt for the Provisional application number will be communicated to applicant by OIPE in due course.

John Dill, Lead Legal Instruments Examiner
Office of Initial Patent Examination

OIPE IAP66
MAR 1 2 2007

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COMMISSIONER FOR PATENTS
UNITED STATES PATENT AND TRADEMARK OFFICE
WASHINGTON, D.C. 20231
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APPLICATION NUMBER	FILING DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	DRAWINGS	TOT CLAIMS	IND CLAIMS
60/331,362	01/04/2001		100	25630/16UTL	6		

CONFIRMATION NO. 2682

23873
ROBERT W STROZIER, PLLC
2925 BRIARPARK, SUITE 930
HOUSTON, TX 77042

FILING RECEIPT

OC000000007733828

Date Mailed: 03/28/2002

Receipt is acknowledged of this provisional Patent Application. It will not be examined for patentability and will become abandoned not later than twelve months after its filing date. Be sure to provide the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION when inquiring about this application. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please write to the Office of Initial Patent Examination's Customer Service Center. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections (if appropriate).**

Applicant(s)

Melanie M. Sohocki, Residence Not Provided;
Stephen P. Daiger, Residence Not Provided;

If Required, Foreign Filing License Granted 03/21/2001

RECEIVED
APR 0 2 2002

Projected Publication Date: Not Applicable

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

Mutations in a novel photoreceptor-pineal gene on 17p cause leber congenital amaurosis (LCA4)

LICENSE FOR FOREIGN FILING UNDER
Title 35, United States Code, Section 184
Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING

LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Office of Export Administration, Department of Commerce (15 CFR 370.10 (j)); the Office of Foreign Assets Control, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

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APPLICATION NUMBER	FILING DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	DRAWINGS	TOT CLAIMS	IND CLAIMS
09/754,842	01/04/2001	1645	0.00	25630/16UTL	6	4	4

01/04/02

CONFIRMATION NO. 2682

23873
ROBERT W STROZIER, PLLC
2925 BRIARPARK, SUITE 930
HOUSTON, TX 77042

FILING RECEIPT

OC0000000005886561

Date Mailed: 03/21/2001

Receipt is acknowledged of this nonprovisional Patent Application. It will be considered in its order and you will be notified as to the results of the examination. Be sure to provide the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION when inquiring about this application. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please write to the Office of Initial Patent Examination's Customer Service Center. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the PTO processes the reply to the Notice, the PTO will generate another Filing Receipt incorporating the requested corrections (if appropriate).**

Applicant(s)

Melanie M. Sohocki, Residence Not Provided;
Stephen P. Daiger, Residence Not Provided;

January 4 2002

Continuing Data as Claimed by Applicant

THIS APPLN CLAIMS BENEFIT OF 60/162,308 10/28/1999

Foreign Applications

If Required, Foreign Filing License Granted 03/21/2001

Projected Publication Date: To Be Determined - pending completion of Missing Parts

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

Mutations in a novel photoreceptor-pineal gene on 17p cause leber congenital amaurosis (LCA4)

R W STROZIER PLLC
DOCKETED

Preliminary Class

530

Data entry by : LE, THANH-LAN **Team :** OIPE **Date:** 03/21/2001

LICENSE FOR FOREIGN FILING UNDER
Title 35, United States Code, Section 184
Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CRF 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 36 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Office of Export Administration, Department of Commerce (15 CFR 370.10 (j)); the Office of Foreign Assets Control, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15 (b).

PLEASE NOTE the following information about the Filing Receipt:

- The articles such as "**a**," "**an**" and "**the**" are not included as the first words in the title of an application. They are considered to be unnecessary to the understanding of the title.
- The words "**new**," "**improved**," "**improvements in**" or "**relating to**" are not included as first words in the title of an application because a patent application, by nature, is a new idea or improvement.
- The title may be truncated if it consists of more than 600 characters (letters and spaces combined).
- The docket number allows a maximum of 25 characters.
- If your application was submitted under 37 CFR 1.10, your filing date should be the "date in" found on the Express Mail label. If there is a discrepancy, you should submit a request for a corrected Filing Receipt along with a copy of the Express Mail label showing the "date in."
- The title is recorded in sentence case.

Any corrections that may need to be done to your Filing Receipt should be directed to:

Assistant Commissioner for Patents
Office of Initial Patent Examination
Customer Service Center
Washington, DC 20231

MAR 12 2007

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Melanie M. **Stephen P.**	**Sohocki** **Daiger**	**Houston, TX** **Houston, TX**

☐ *Additional inventors are being named on the __ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (280 characters max)

MUTATIONS IN A NOVEL PHOTORECPTOR-PINEAL GENE ON 17p CAUSE LEBER CONGENITAL AMAUROSIS (LCA4)

Direct all correspondence to:

☒ Customer Number 23873

OR Customer Number here Type

☐ Firm or Individual Name

Address					
City		State		ZIP	
Country		Telephone		Fax	

ENCLOSED APPLICATION PARTS (*check all that apply*)

☒ Specification 15
☒ Sheet of Drawings 6
☐ Application Data Sheet. See 37 CFR 1.76
☐ CD(s), Number
☒ Other (specify) Postcard

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☒ Applicant claims small entity status. See 37 CFR 1.27.
☒ A check or money order is enclosed to cover the filing fees.
☒ The Commissioner is hereby authorized to charge the filing fee to Deposit Account Number: **501518**.
☒ The Commissioner is hereby authorized to charge any deficiencies or credit any overpayment to Deposit Account Number: **501518**.
☐ Payment by credit card. Form PCT-2038 is attached.

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☐ No
☒ Yes, the name of the U.S. Government agency and the Government contract number are: EY07142 NEI/NIH

Respectfully submitted,

SIGNATURE _____ Date: **4 January 2001**

NAME **Robert W. Strozier** REGISTRATION NO. **34,024**

TELEPHONE **713.977.7000** Docket No. **96606/16PRV**

OIPE IAP66
MAR 1 2 2007
PATENT & TRADEMARK OFFICE

UNITED STATES PATENT APPLICATION

TITLE: **MUTATIONS IN A NOVEL PHOTORECEPTOR-PINEAL GENE ON 17P CAUSE LEBER CONGENITAL AMAUROSIS (LCA4)**

INVENTOR: Melanie M. Sohocki & Stephen P. Daiger EL389348319US

GOVERNMENTAL SUPPORT

The invention disclosed herein was developed impart from funds from grant EY07142 from the National Eye Institute-National Institutes of Health (M.M.S., S.J.B., L.S.S. and S.P.D.).

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a novel a novel photoreceptor/pineal-expressed gene encoding aryl-hydrocarbon interacting protein-like 1 (*AIPL1*).

More particularly, the present invention relates to

2. Description of the Related Art

Leber congenital amaurosis (LCA, MIM 204000) accounts for at least 5% of all inherited retinal disease (Kaplan J., Bonneau D., Frezal J., Munnich A. & Dufier J.L. Clinical and genetic heterogeneity in retinitis pigmentosa. *Hum. Genet.* **85**, 635-642 (1990)), and is the most severe inherited retinopathy, with the earliest age of onset (Foxman, S.G., Heckenlively, J.R., Batemen, B.J. & Wirstschafter, J.D. Classification of congenital and early-onset retinitis pigmentosa. *Arch. Ophthalmol.* **103**, 1502-1507 (1985)). LCA is diagnosed at birth or in the first few months of life, with severely impaired vision or blindness, nystagmus, and a markedly abnormal or flat electroretinogram (ERG). Mutations in *GUCY2D* (Perrault, I. *et al.* Retinal-specific guanylate cyclase gene mutations in Leber's congenital amaurosis. *Nature Genet.* **14**, 461-464 (1996)), *RPE65* (Marlhens, F. *et al.* Mutations in RPE65 cause Leber's congenital amaurosis. *Nature Genet.* **17**, 139-141 (1997))and *CRX* (Freund, C.L. *et al. De novo* mutations in the CRX homeobox gene associated with Leber congenital amaurosis. *Nature Genet.* **18**, 311-312 (1998)) are known

to cause LCA. However, one study identified disease-causing *GUCY2D* mutations in only 8 of 15 families whose LCA locus maps to 17p13.1 (Perrault, I. *et al.* Retinal-specific guanylate cyclase gene mutations in Leber's congenital amaurosis. *Nature Genet.* **14**, 461-464 (1996)), suggesting another LCA locus might be located on 17p13.1. Confirming this prediction, the LCA in one Pakistani family mapped to 17p13.1, between *D17S849* and *D17S960*—a region that *excludes GUCY2D*. The LCA in this family has been designated LCA4 (Hameed, A. *et al.* A novel locus for Leber congenital amaurosis with anterior keratoconus mapping to 17p13. *Invest. Ophthalmol. Vis. Sci.* in press (1999)).

SUMMARY OF THE INVENTION

The present invention provides a new photoreceptor/pineal-expressed gene, aryl-hydrocarbon interacting protein-like 1 (*AIPL1*), which maps within the LCA4 candidate region, and comprises three tetratricopeptide (TPR) motifs. These motifs are thought to be consistent with nuclear transport or chaperone activity.

The present invention provides a method for identifying photoreceptor/pineal-expressed gene, aryl-hydrocarbon interacting protein-like 1 (*AIPL1*) including specific mutations that give rise to LCA.

The present invention also provides a anti-sense base sequences capable of binding to and allow identification of mutant genes.

DESCRIPTION OF THE DRAWINGS

The invention can be better understood with reference to the following detailed description together with the appended illustrative drawings in which like elements are numbered the same:

Figure 1 depicts a gene and protein structure of *AIPL1*;

Figure 2 depicts a fluorescence *in situ* hybridization (FISH);

Figure 3 depicts an expression of AIPL1 in human tissues;

Figure 4 depicts a retina and pineal expression of *Aipl1*;

Figure 5 depicts a pedigrees and mutation screen of AIPL1 in families;

Figure 6 depicts a fundus photograph of affected LCA patient (eleven years of age), displaying typical symptoms of Leber congenital amaurosis; widespread retinal pigment epithelium changes with pigment clumping, attenuated retinal vessels, pale optic disk, and macular atrophy are evident. Members of the KC family also display keratoconus; because *AIPL1* is not expressed in the cornea, it is possible that this symptom is secondary to LCA in this family, due to eye rubbing, *etc.*

DETAILED DESCRIPTION OF THE INVENTION

The inventors have found that a homozygous nonsense mutation at codon 278 is present in all affected members of the original LCA4 family. *AIPL1* mutations may cause approximately 20% of recessive LCA, as disease-causing mutations were identified in 3 of 14 LCA families not tested previously for linkage.

In a previous study, STSs designed to the retina/pineal-expressed EST clusters THC220430 and THC90422 originally mapped to 17p13 (Sohocki, M.M., Malone, K.A., Sullivan, L.S. & Daiger, S.P. Localization of retina/pineal – expressed sequences (ESTs): identification of novel candidate genes for inherited retinal disorders. *Genomics* **58**, 29-33 (1999)), near a retinitis pigmentosa (RP13) candidate region (Greenberg, J., Goliath, R., Beighton, P., & Ramesar, R. A new locus for autosomal dominant retinitis pigmentosa on the short arm of chromosome 17. *Hum Mol Genet* **3**, 915-918 (1994)). Further testing refined the localization to 17p13.1, between SHGC-2251 and SHGC-6095, within the LCA4 candidate region, and approximately 2.5 megabases (Mb) distal to *GUCY2D*. Fluorescence *in situ* hybridization as shown in Figure 2 confirmed the localization. *AIPL1*-containing bacterial artificial chromosome (BAC), shown in red, hybridizes to 17p13.1, consistent with placement of *AIPL1* in the Stanford G3 radiation hybrid panel. These data refute the original placement of *AIPL1* to 17p13.3 by placement in the GeneBridge 4.0 radiation hybrid panel. Chromosome 17 alpha-satellite DNA is indicated in green.

cDNA sequencing of the two clusters indicated that the ESTs represent transcripts of one gene. THC90422 transcripts bypass the THC220430 polyadenylation signal, resulting in a 709 bp longer 3' untranslated region (UTR). The 180 bp 5' UTR and coding sequence

encoded by the six-exon gene are identical in the 1538 bp and 2247 bp transcripts as shown in Figure 1a. *AIPL1* comprises six exons, with alternate polyadenylation sites in the 3' untranslated region, shown by arrows. Cys239Arg denotes the location of the TGC→CGC missense mutation in exon 5 of the RFS128 family. Trp278X denotes the location of the TGG→TGA nonsense mutation in exon 6 of the KC, MD, RFS127 and RFS121 families. Ala336Δ2 denotes the location of the 2 bp deletion in exon 6 of RFS121. Benign coding sequence substitutions identified were Phe37Phe (TTT/TTC; 0.98/0.02 frequency), Cys89Cys (TGC/TGT; 0.99/0.01), Asp90His (GAC/CAC; 0.84/0.16), Leu100Leu (CTG/CTA; 0.57/0.43) and Pro217Pro (CCG/CCA; 0.61/0.39).

The gene was named human aryl hydrocarbon receptor-interacting protein-like 1 (*AIPL1*) due to extensive similarity (49% identity, 69% positive) to human aryl hydrocarbon receptor-interacting protein (AIP), a member of the FK506-binding protein (FKBP) family (Ma, Q. & Whitlock Jr., J.P. A novel cytoplasmic protein that interacts with the Ah receptor, contains tetratricopeptide repeat motifs, and augments the transcriptional response to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *J. Biol. Chem.* **272**, 8878-8884 (1997)) as shown in Figure 1b. The protein sequence of AIPL1 alignment demonstrates the high level of sequence conservation between rat and human AIPL1, and mouse and human AIP. Identical residues in the four sequences are noted with an asterisk; identical residues in three of the sequences are indicated with a period. The predicted protein comprises 384 amino acids, with a 43,865 Dalton molecular mass, and a 5.57 pI. The protein sequence includes three tetratricopeptide repeats (TPR), a 34 amino acid motif found in proteins with nuclear transport or protein chaperone activity (Ma, Q. & Whitlock Jr., J.P. A novel cytoplasmic protein that interacts with the Ah receptor, contains tetratricopeptide repeat motifs, and augments the transcriptional response to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *J. Biol. Chem.* **272**, 8878-8884 (1997)).

Northern hybridization identified mRNA molecules of the predicted sizes in total retinal RNA. The probe also cross-hybridized to 18s rRNA as shown in Figure 3 in the retina. As shown in Figure 3, the Northern blots from adult tissues were incubated with an

AIPL1 probe. Total retinal RNA blot, exposed 4 hours at – 70°C (upper left) and polyA$^+$ RNA multi-tissue Northern (MTN), exposed 72 hours at – 70°C (upper right). No signal was observed in MTN at 4, 24, or 48-hour exposure. Lane 1, adult retina; lane 2, heart; lane 3, whole brain; lane 4, placenta; lane 5, lung; lane 6, liver; lane 7, skeletal muscle; lane 8, kidney; lane 9, pancreas. Both blots were incubated with a β-actin probe as a control (lower panel). Solid arrows indicate mRNA molecules of the predicted sizes, 1538 and 2247 bp, in retina. A weaker signal was detected in skeletal muscle and heart on a polyA$^+$ RNA multi-tissue Northern after very long exposure. It is likely that this signal represents cross-hybridization, as the transcripts differ in size from the retinal mRNAs, and are faint. The Northern did not indicate *AIPL1* expression in brain; however, only cerebral tissue was included in the blot. *In situ* hybridization indicated expression in rat and mouse pineal, a high level of expression in adult mouse photoreceptors as shown in Figure 4, and no expression in cornea (data not shown). As shown in Figure 4a, digoxygenin *in situ* hybridization of Aipl1 in adult mouse retina, with expression throughout the outer nuclear layer and photoreceptor inner segments. Color reaction time is 4 days. As shown in Figure 4b, sense control of "a" with same reaction time. A slight background signal is observed across photoreceptor outer segments.As shown in Figure 4c, short (16 hour) color reaction of *Aipl1* in adult mouse retina, showing a high level of mRNA in photoreceptor inner segments. As shown in Figure 4d, expression of *Aipl1* in adult mouse pineal. Color reaction time is 4 days. As shown in Figure 4e, Sense control of "d", with same reaction time. As shown in Figure 4f, expression of *Aipl1* mRNA in P14 rat pineal. Color reaction time is 4 days. As shown in Figure 4g, sense control of "f", with same reaction time. Scale bar for a-c is 30 μm, for d and e is 50 μm, and f and g is 70 μm. RPE-retinal pigment epithelium, OS-outer photoreceptor segment, IS-inner photoreceptor segment, ONL-outer nuclear layer, INL-inner nuclear layer, GCL-ganglion cell layer. Immunolocalization of the AIPL1 protein has not been performed; therefore, site of AIPL1 protein localization is currently unknown.

Sequencing of the rat *Aipl1* cDNA revealed extensive amino acid sequence conservation (87% identity and 96% similarity) between rat and human *AIPL1*. Interestingly,

rat *Aipl1*, mouse Aip, and human AIP lack a 56 amino acid carboxyl-terminal extension present in *AIPL1* as shown in Figure 1b; this extension includes a "hinge" motif of high flexibility, with multiple O-glycosylation sites, and a casein kinase II (CK2) phosphorylation site, which is thought to be involved in protein complex regulation, as is the CK2 site within the hinge of another FKBP family member, FKBP52 (Miyata, Y. et al. Phosphorylation of the immunosuppressant FK506-binding protein FKBP52 by casein kinase II: regulation of HSP90-binding activity of FKBP52. *Proc. Natl. Acad. Sci.* **94**, 14500-14505 (1997)). The hinge appears to be conserved in primates, as it is also present in the squirrel monkey (*Saimiri sciureus*; data not shown).

Single-stranded conformational analysis (SSCA) identified three benign nucleotide substitutions within the *AIPL1* exon 3 amplimer: G/A at – 14, G/A at – 10 bp, and G/A at codon 100 (Leu100Leu, CTG/CTA). Four haplotypes were identified for the combined polymorphisms; the most common, GCG and GAA, have frequencies of 55% and 41%, respectively.

Referring now to Figure 5, pedigrees and mutation screen of *AIPL1* in families is shown. As shown in Figure 5a, the Trp278X mutation is homozygous in three families: KC, MD and RFS127. SSCA of all living individuals of the KC pedigree demonstrate segregation of the mutant allele. Top electropherogram: an unaffected control (TGG/TGG). Middle: heterozygous G/A mutation at codon 278. Bottom: DNA sequence of a homozygous, affected member of MD (TGA/TGA). As shown in Figure 5b, the RFS121 affected individuals are compound heterozygotes for the Trp278X and Ala336Δ2 bp mutations. Top electropherogram: unaffected control, bottom: heterozygous G/A mutation at codon 278 (left) and heterozygous 2 bp deletion beginning in codon 336 (right) in an affected individual of RFS121. As shown in Figure 5c, the Cys239Arg mutation found in family RFS128. Top electopherogram: unaffected control (TGC/TGC), bottom: DNA sequence of a homozygous, affected individual (CGC/CGC).

Sequencing of *AIPL1* from the DNA of one affected individual of the original LCA4 family as shown in Figure 5a, revealed a homozygous nonsense mutation (Trp278X,

TGG→TGA). This allele, if expressed, encodes a protein 107 amino acids shorter than wild-type *AIPL1*. The truncated protein includes only 20 of the 34 amino acids of the third TPR motif, a region conserved between human, rat and mouse *AIPL1*, and AIP. SSCA in other family members confirmed that all affected family members are homozygous for this mutation as shown in Figure 5a, and that 100 ethnically-matched controls did not carry this mutation.

AIPL1 was next analyzed in another Pakistani family, MD as shown in Figure 5a, whose LCA had been mapped to 17p13.1, with *GUCY2D* excluded by mutational analysis. Sequencing of *AIPL1* indicated that affected individuals of this family are homozygous for the Trp278X mutation as shown in Figure 5a. The MD and KC families differ in haplotype (GCG and GAA, respectively) of the *AIPL1* exon 3 polymorphisms, as well as for microsatellite markers tightly linked to *AIPL1*. These results are though to suggest that the Trp278X mutations causing the LCA in these two families are not derived from a recent, common ancestor.

Assay of *AIPL1* in fourteen Caucasian families with LCA that had not been tested previously for linkage to 17p identified apparent disease-causing mutations in three additional families, as follows.

Family RFS121

Direct sequencing of *AIPL1* in the two affected RFS121 individuals indicated two mutations, a 2 bp deletion in codon 336 (Ala336Δ2 bp; see Figure 5b) and the Trp278X mutation. The deletion results in a frame shift and a termination delayed by 47 codons. The termination signal used in the deletion transcript is upstream of the first *AIPL1* polyadenylation signal; therefore, the alternate transcripts from this allele are not predicted to encode alternate proteins. Allele-specific PCR in one affected individual confirmed that the 2 bp deletion and Trp278X mutations are on opposite chromosomes. Therefore, the affected individuals in RFS121 are compound heterozygotes, having received the Trp278X mutation from one parent and the Ala336Δ2 mutation from the other. No unaffected RFS121 family members inherited both mutations. The Ala336Δ2 bp mutation was not

observed in 55 unrelated Caucasian control individuals.

Family RFS127

AIPL1 sequencing from two affected RFS127 individuals as shown in Figure 5a indicated homozygous Trp278X mutations — the same mutation identified in KC and MD. Haplotype analysis of tightly linked microsatellite markers, and of the *AIPL1* exon 3 polymorphisms suggest that the mutations in the RFS127 and MD families are likely to have descended from a common ancestor. However, there is no indication of Pakistani origin for members of this family.

Family RFS128

The three affected individuals of RFS128 as shown in Figure 5c are homozygous for a T→C nucleotide substitution predicted to encode a Cys239Arg substitution. This cysteine is conserved in human and rat AIPL1, and in AIP as shown in Figure 1. This mutation was not identified in over 55 ethnically-matched control individuals. Affected members of this family are homozygous for microsatellite markers *D17S796* and *D17S1881*, tightly linked, flanking markers of *AIPL1*. In contrast, affected family members are heterozygous for microsatellite markers *D17S960* and *D17S1353*, which flank *GUCY2D*.

These findings indicate that we have identified a novel gene that causes LCA4, having detected homozygous *AIPL1* mutations in three families in which *GUCY2D* was *excluded* as the cause of the disease by linkage and/or mutation screening: KC, MD and RFS128. *AIPL1* is the fourth gene to be associated with LCA. Mutations in *AIPL1* may be a common cause of LCA, as an *AIPL1* mutation was identified as the apparent cause of the retinal disease in three of fourteen (21 ± 8%, 90% C.I.) unmapped LCA families. *AIPL1* should be assayed in LCA families whose disease locus maps to 17p13, but with no apparent disease-causing mutations in *GUCY2D*, as in 7 of the 15 original LCA1 families (Marlhens, F. *et al.* Mutations in RPE65 cause Leber's congenital amaurosis. *Nature Genet.* **17**, 139-141 (1997)). Due to the proximity of *AIPL1* and *GUCY2D* on 17p13, linkage mapping may not distinguish between the genes. Further, it is possible that LCA patients who are identical by descent (IBD) at one locus are also IBD at the other. Therefore, both *AIPL1* and *GUCY2D* should

be screened for mutations in families whose LCA locus maps to 17p13 or in families with affected individuals who are homozygous for mutations in either gene, unless linkage excludes one of the genes. Of the five families reported here, *GUCY2D* was excluded by linkage testing and/or mutation screening in three; the fourth is a compound heterozygote; and the fifth is homozygous for a disease-causing mutation confirmed in other families.

The similarity of *AIPL1* to AIP and the presence of three TPR motifs suggest that it may be involved in retinal protein folding and/or trafficking. Its role in the pineal gland is also uncertain. The pineal gland contributes to resetting circadian rhythm by diurnal release of melatonin. Additionally, children with destructive pinealomas often display precocious puberty, suggesting a role in long-term periodicity (Endocrine role of the pineal gland. in *Endocrinology* (ed. Hadley, M.E.) 458-476 (New York, New York, 1996)). Because LCA patients with *AIPL1* mutations have grossly abnormal photoreceptors at an early age, the pineal gland also may be affected. Careful clinical characterization of LCA4 patients may reveal pineal-associated abnormalities. Therefore, identifying the exact role of *AIPL1* in photoreceptors and the pineal gland will improve our understanding of disease pathology in these patients, and contribute to our understanding of the biology of normal vision and pineal activity.

METHODS

cDNA sequencing and RACE

The inventors obtained partial cDNA clones for THC220430 (fetal retina IMAGE 838161, adult retina ATCC 117797, pineal gland IMAGE 232323) and THC90422 (adult retina: ATCC 11795, pineal gland: ATCC 170258, IMAGE 383092) from Research Genetics or ATCC and purified them using the QIAprep spin miniprep kit (QIAGEN). The inventors, then sequenced cDNAs using a primer- walking technique, with the AmpliCycle sequencing kit (Perkin Elmer), and ^{32}P-labeled primers, beginning with M13 vector primers. Using the human retina Marathon-ready cDNA (Clontech) and the Marathon RACE kit (Clontech), RACE identified the 5' untranslated region of AIPL1 and obtained the polyadenylation signal of the THC90422 transcripts.

Northern Blot Analysis

The inventors probed a human multiple tissue polyA$^+$ RNA Northern blot and a human adult retina total RNA Northern blot at the same time with an amplimer from exon 6 and the 3' untranslated region of *AIPL1* that was ^{32}P-labeled using the Strip-EZ PCR kit (Ambion). Hybridization was in ULTRA-hyb solution (Ambion) according to the manufacturer's protocols. As a positive control, the inventors incubated both blots with human β-actin using the same reaction conditions.

Retinal/pineal *in situ* hybridization

PCR of a mouse retinal cDNA library using PCR primers designed to the human *AIPL1* cDNA (5'-AAGAAAACCATTCTGCACGG-3' and 5'-TGCAGCTCGTCCAGGTCCT-3') obtained a 613 bp fragment of mouse *AIPL1* cDNA. Sequencing of the resulting fragment using the AmpliCycle Sequencing kit (Perkin Elmer) and ^{32}P end-labeled primers confirmed that the resulting fragment represented mouse *Aipl1* cDNA. The fragment was used as a probe for digoxygenin *in situ* hybridization using previously described methods (Furukawa, T., Morrow, E.M. & Cepko, C.L. *Crx*, a novel *otx*-like homeobox gene, shows photoreceptor-specific expression and regulates photoreceptor differentiation. *Cell* **91**, 531-541 (1997)).

Genomic sequencing of BAC clones

The Human BAC I library was screened commercially (Genome Systems) using PCR primer pairs based on the *AIPL1* sequence (5'-GACACCTCCCTTTCTCC-3' and 5'-GCTGGGGCTGCCTGGCTG-3'; 5'-CCGAGTGATTACCAGAGGGA-3' and 5'-TGAGCTCCAGCACCTCATAG-3'). The inventors purified BAC DNA from the identified clones using the Plasmid Midiprep Kit (QIAGEN) and sequenced it directly using an ABI310 automated sequencer. A primer walking strategy beginning with PCR primers to the cDNA obtained complete intronic sequences. The inventors viewed, edited and aligned sequence data using AutoAssembler (Perkin Elmer) software.

Fluorescence *In situ* Hybridization

Fluorescence *In situ* Hybridization (FISH) was performed on normal human

chromosome slides prepared by standard cytogenetic procedures. BAC264k12 was labeled with digoxygenin (Boehringer Mannhiem) by nick translation and a probe consisting of 200 ng labeled BAC DNA, 10 μg salmon sperm DNA, 5 μg Human Cot-1 DNA (Gibco BRL) and chromosome 17 alpha satellite DNA labeled with Spectrum Green (Vysis) was denatured and hybridized to denatured slides. Unbound probe was removed by washing in 72°C 1 X SSC buffer and the digoxygenin-labeled DNA was detected with anti-digoxygenin rhodamine (Boehringer Mannheim). Chromosomes were counterstained with 0.2 μg.ml DAPI in an anti-fade solution. Images were captured using the PowerGene probe analysis system (Perceptive Scientific Instruments Inc.).

Radiation Hybrid Panel Mapping

PCR of the STSs originally designed to EST clusters THC220430 and THC90422 in the Stanford G3 radiation hybrid panel confirmed the chromosomal location of *AIPL1*. The Stanford Human Genome Center RHServer (http://www-shgc.stanford.edu/RH/) interpreted data for chromosomal location.

Patients and Families

All patients gave informed consent prior to their participation in this study. For each case, clinical evaluation was by at least one of the coauthors.

All affected individuals of the original LCA4 family, KC, are affected with Leber congenital amaurosis and bilateral keratoconus. Clinical examination of the affected individuals revealed bilateral ectasia with central thinning of the cornea, before they reached their twenties. The central cornea has a pronounced cone shape with severe corneal clouding. All affected individuals were blind from birth, with absence of rod and cone function as demonstrated by ERG. Patients also show pigmentary deposits in the retina.

All affected individuals of family MD were blind from birth with absence of rod and cone function as demonstrated by ERG, but without keratoconus. Fundus examination indicates pigmentary retinopathy, attenuated blood vessels, and marked macular degeneration.

The two affected individuals of RFS121 had poor central vision from birth, along with

severe night blindness and pendular nystagmus. Fundus examination revealed widespread retinal pigment epithelium changes with pigment clumping in the far periphery, severely attenuated retinal vessels, pronounced atrophy within the macula and a pale optic disk. ERG testing in the third decade of life showed non-detectable cone and rod responses.

Affected individuals in family RFS127 also had poor central vision from birth, severe night blindness and pendular nystagmus. Full-field ERGs in the second decade of life revealed non-detectable responses to all stimuli. Fundus examination revealed widespread retinal pigment epithelial changes with pigment clumping, attenuated retinal vessels, macular atrophy and a pale optic disk.

All affected individuals of RFS128 displayed poor central vision from birth, severe night blindness and pendular nystagmus. Cone ERGs to 31 Hz flicker were non-detectable during the first decade of life. A response up to 15 μV to a maximal stimulus flash (presumably rod-mediated) was present during the first decade but borderline detectable by the second decade. Widespread pigment epithelium changes with pigment clumping, attenuated retinal vessels, macular atrophy and pale optic disks were present in affected family members as shown Figure 6.

Mutation Analysis and Genotyping

The inventors performed direct sequencing for initial mutation analysis, sequencing PCR-amplified *AIPL1* exons using a BigDye terminator sequencing kit (Perkin Elmer) on an ABI 310 automated sequencer, according to the manufacturer's protocols.

The inventors performed allele-specific PCR in RFS121 using PCR primers specific to *AIPL1* exon 6 sequence, with the forward primer annealing specifically to the wild-type sequence for codon 278 (5'-ACGCAGAGGTGTGGAATG-3') and the reverse primer in the 3' untranslated sequence (5'-AAAAAGTGACACCACGATC-3'). The inventors sequenced PCR products as described above.

Primer pairs for microsatellite markers were obtained from Research Genetics. The forward-strand primer was end-labeled with ^{32}P and polynucleotide kinase (Promega). Amplification, product separation, and visualization were as described previously (Perrault,

I. *et al.* Retinal-specific guanylate cyclase gene mutations in Leber's congenital amaurosis. *Nature Genet.* **14,** 461-464 (1996)). Single stranded conformational analysis was performed at room temperature and 4°C by previously reported methods (Perrault, I. *et al.* Retinal-specific guanylate cyclase gene mutations in Leber's congenital amaurosis. *Nature Genet.* **14,** 461-464 (1996)), using directly-sequenced individuals as controls.

Genbank Accession Numbers

Human *AIPL1* cDNA and primer sequences, AF148864; mouse *Aipl1* partial cDNA sequence AF151392; complete rat *Aipl1* cDNA sequence AF180340; partial squirrel monkey *Aipl1* genomic sequence AF180341; human genomic *AIPL1* sequence AF180472.

All references cited herein are incorporated by reference. While this invention has been described fully and completely, it should be understood that, within the scope of the appended claims, the invention may be practiced otherwise than as specifically described. Although the invention has been disclosed with reference to its preferred embodiments, from reading this description those of skill in the art may appreciate changes and modification that may be made which do not depart from the scope and spirit of the invention as described above and claimed hereafter.

CLAIMS

We claim:

1. A photoreceptor/pineal-expressed gene, aryl-hydrocarbon interacting protein-like 1 (*AIPL1*) within the LCA4 region comprising three tetratricopeptide (TPR) motifs.

2. A method for identifying photoreceptor/pineal-expressed gene, aryl-hydrocarbon interacting protein-like 1 (*AIPL1*) including specific mutations that give rise to LCA comprising sequencing of the AIPL1 gene encoding region of a patiences DNA and determining with the AIPL1 encoding region includes a specific mutation.

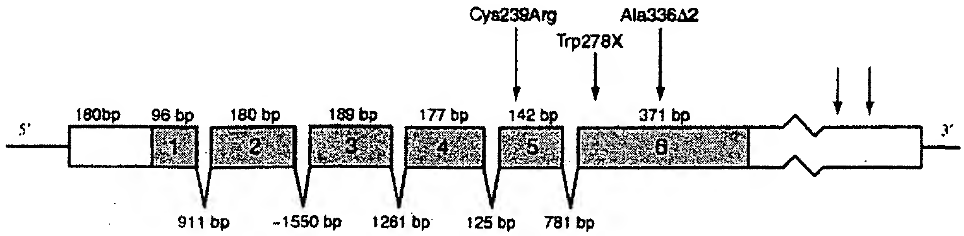
3. An anti-sense base sequences capable of binding to and allow identification of mutant genes comprising an anti-sense sequence of base comprising all or a part of mutant AILP1 encoding regions.

4. A protein comprising the sequence of amino acids of Figure 1.

ABSTRACT

A novel photoreceptor/pineal-expressed gene encoding aryl-hydrocarbon interacting protein-like 1 (*AIPL1*), the associated protein like amino acid sequence and methods for identifying the presence of the sequence in patients.

a.



b.

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Human AIPL1   .MDAALLLNV EGVKKTILHG GTGELPNFIT GSRVIFHFRT MKCDEERTVI
Rat AIPL1     .MDVSLLLNV EGVKKTILHG GTGELPNFIT GSRVTFHFRT MKCDEERTVI
Human AIP     MADIIARLRE DGIQKRVIQE GRGELPDFQD GTKATFHYRT LHSDDEGTVL
Mouse AIP     MADLIARLRE DGIQKRVIQE GRGELPDFQD GTKATFHFRT LHSDNEGSVI
                                                                  100
Human AIPL1   DDSRQVGQPM HIIIGNMFKL EVWEILLTSM RVHEVAEFWC DTIHTGVYPI
Rat AIPL1     DDSKQVGQPM NIIIGNMFKL EVWETLLTSM RLGEVAEFWC DTIHTGVYPM
Human AIP     DDSRARGKPM ELIIGKKFKL PVWETIVCTM REGEIAQFLC DIKHVVLYPL
Mouse AIP     DDSRTRGKPM ELIVGKKFKL PVWETIVCTM REGEIAQFLC DIKHVVLYPL
                                                TPR I
Human AIPL1   LSRSLRQMAQ GKDPTEWHVH TCGLANMFAY HTLGYEDLDE LQKEPQPLVF
Rat AIPL1     LSRSLRQVAE GKDPTSWHVH TCGLANMFAY HTLGYEDLDE LQKEPQPLIF
Human AIP     VAKSLRNIAV GKDPLEGQRH CCGVAQMREH SSLGHADLDA LQQNPQPLIF
Mouse AIP     VAKSLRNIAE GKDPLEGQRH CCGIAQMHEH SSLGHADLDA LQQNPQPLIF
                                                                  200
Human AIPL1   VIELLQVDAP SDYQRETWNL SNHEKMKAVP VLHGEGNRLF KLGRYEEASS
Rat AIPL1     LIKLLQVEAP NEYQRETWNL NNEERMQAVP LLHGEGNRLY KLGRYDQAAT
Human AIP     HMEMLKVESP GTYQQDPWAM TDEEKAKAVP LIHQEGNRLY REGHVKEAAA
Mouse AIP     HIEMLKVESP GTYQQDPWAM TDEKAKAVP VIHQEGNRLY REGQVKEAAA
                                     TPR II
Human AIPL1   KYQEAIICLR NLQTKEKPWE VQWLKLEKMI NTLILNYCQC LLKKEEYYEV
Rat AIPL1     KYQEAIVCLR NLQTKEKPWE VEWLKLEKMI NTLILNYCQC LLKKEEYYEV
Human AIP     KYYDAIACLK NLQMKEQPGS PEWIQLDKQI TPLLLNYCQC KLVVEEYYEV
Mouse AIP     KYYDAIACLK NLQMKEQPGS PDWIQLDLQI TPLLLNYCQC KLVAQEYYEV
                                        TPR III                   300
Human AIPL1   LEHTSDILRH HPGIVKAYYV RARAHAEVWN EAEAKADLQK VLELEPSMQK
Rat AIPL1     LEHTSDILRH HPGIVKAYYM RARAHAEVWN AEEAKADLEK VLELEPSMRK
Human AIP     LDHCSSILNK YDDNVKAYFK RGKAHAAVWN AQEAQADFAK VLELDPALAP
Mouse AIP     LDHCSSILNK YDDNVKAYFK RGKAHAAVWN AQEAQADFAK VLELDPALAP

Human AIPL1   AVRRELRLLE NRMAEKQEEE RLRCRNMLSQ GATQPPAEPP TEPPAQSSTE
Rat AIPL1     AVLRELRLLE SRLADKQEEE RQRCRSMLG. .......... ..........
Human AIP     VVSRELRALE ARIRQKDEED KARFRGIFSH .......... ..........
Mouse AIP     VVSRELRALE TRIRQKDEED KARFRGIFSH .......... ..........

Human AIPL1   PPAEPPTAPS AELSAGPPAE PATEPPPSPG HSLQH
Rat AIPL1     .......... .......... .......... .....
Human AIP     .......... .......... .......... .....
Mouse AIP     .......... .......... .......... .....
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Fig. 1 Gene and protein structure of AIPL1. ***a.*** *AIPL1* consists of six exons, with alternate polyadenylation sites in the 3′ untranslated region, shown by arrows. Cys239Arg denotes the location of the TGC→CGC missense mutation in exon 5 of the RFS128 family. Trp278X denotes the location of the TGG→TGA nonsense mutation in exon 6 of the KC, MD, RFS127 and RFS121 families. Ala336Δ2 denotes the location of the 2 bp deletion in exon 6 of RFS121. Benign coding sequence substitutions identified were Phe37Phe (TTT/TTC; 0.98/0.02 frequency), Cys89Cys (TGC/TGT; 0.99/0.01), Asp90His (GAC/CAC; 0.84/0.16), Leu100Leu (CTG/CTA; 0.57/0.43) and Pro217Pro (CCG/CCA; 0.61/0.39) ***b.*** Protein sequence of AIPL1. The alignment demonstrates the high level of sequence conservation between rat and human AIPL1, and mouse and human AIP. Identical residues in the four sequences are noted with an asterisk; identical residues in three of the sequences are indicated with a period.

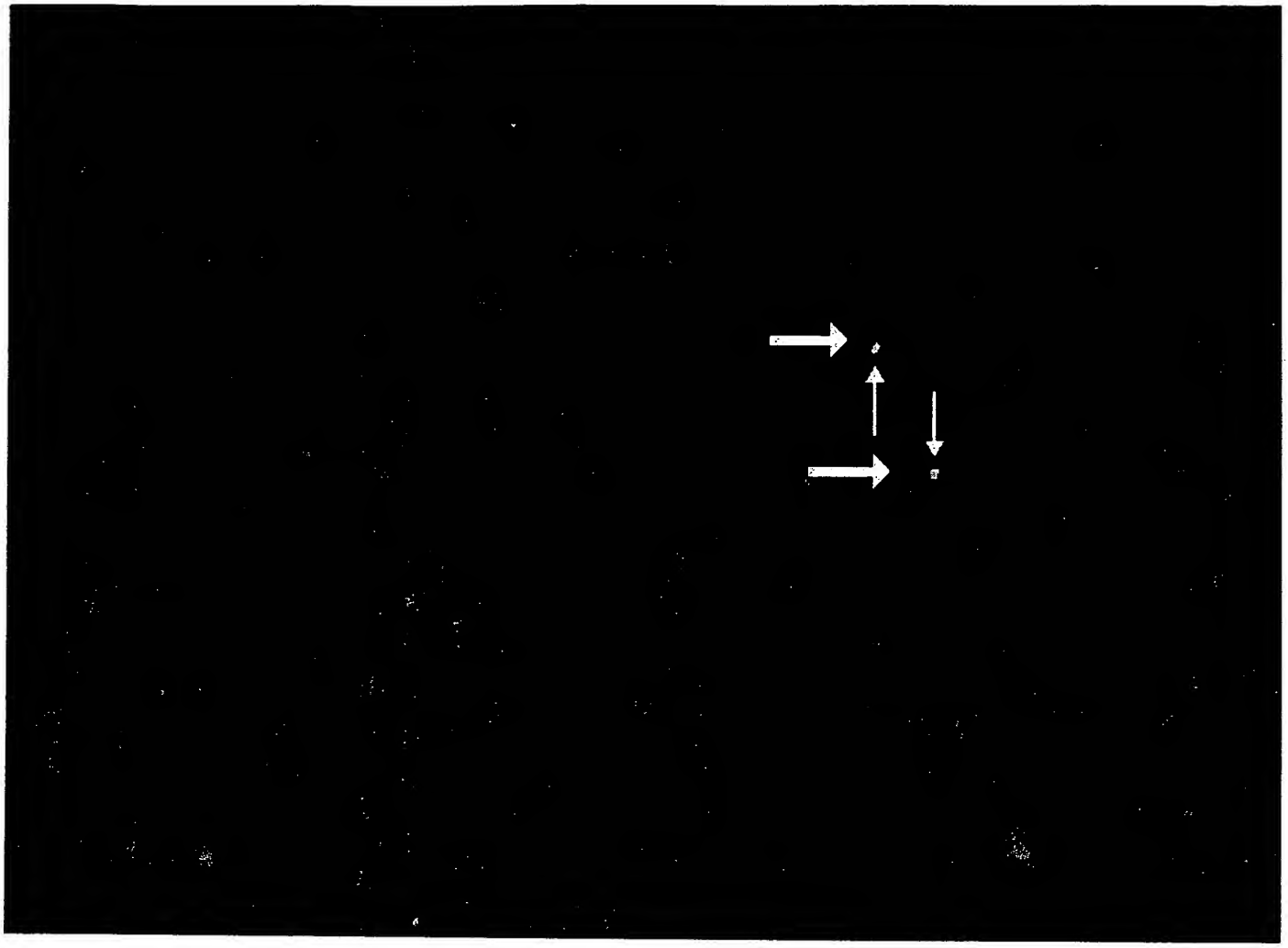

Fig. 2 Fluorescence *in situ* hybridization (FISH). *AIPL1*-containing bacterial artificial chromosome (BAC), shown in red, hybridizes to 17p13.1, consistent with placement of *AIPL1* in the Stanford G3 radiation hybrid panel. These data refute the original placement of *AIPL1* to 17p13.3 by placement in the GeneBridge 4.0 radiation hybrid panel. Chromosome 17 alpha-satellite DNA indicated in green.

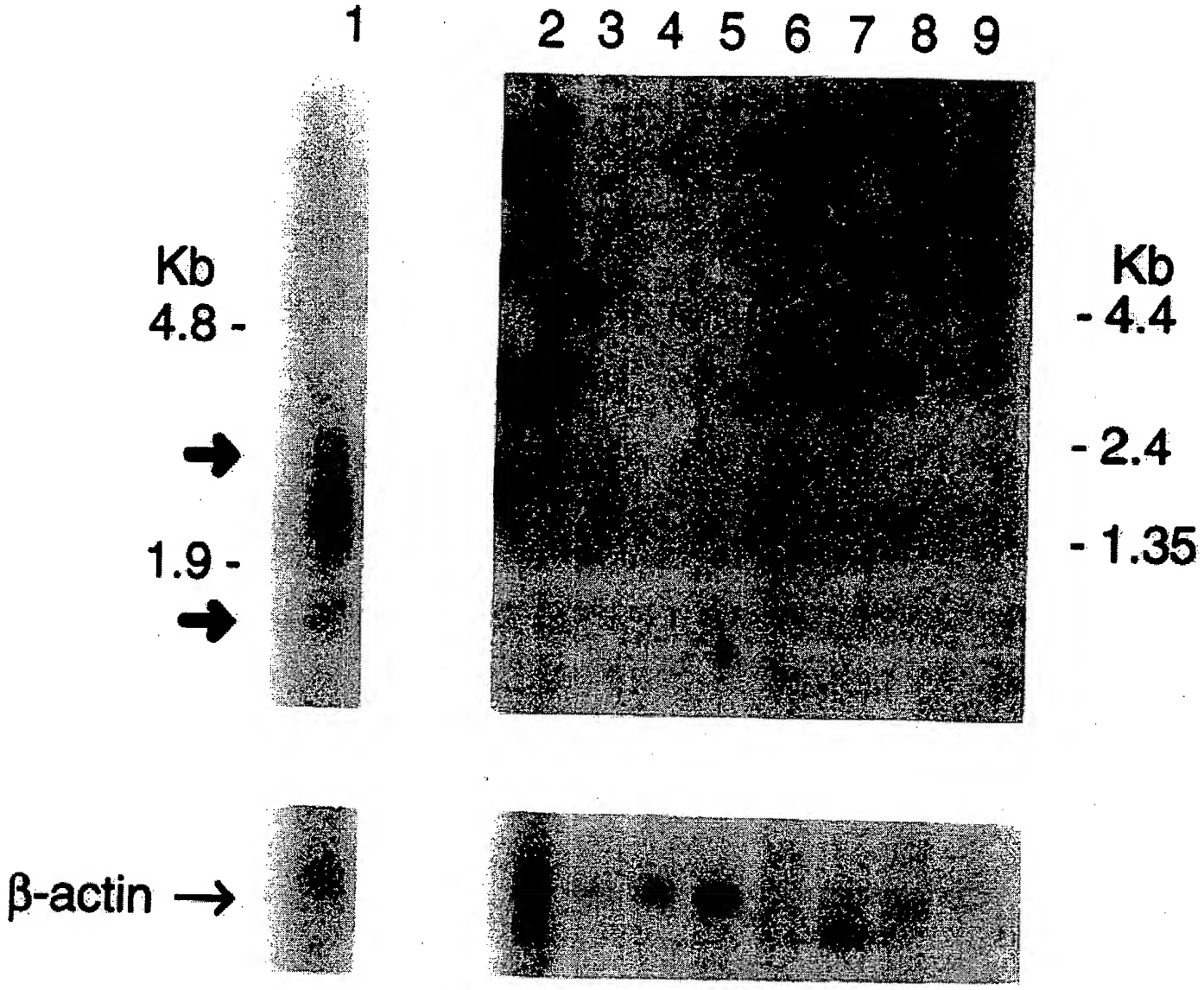

Fig. 3 Expression of AIPL1 in human tissues. Nothern blots from adult tissues were incubated with an AIPL1 probe. Total retinal RNA blot, exposed 4 hours at –70 °C (upper left) and polyA$^+$ RNA multi-tissue Northern (MTN), exposed 72 hours at –70 °C (upper right). No signal was observed in MTN at 4, 24, or 48 hour exposure. Lane 1, adult retina; lane 2, heart; lane 3, whole brain; lane 4, placenta; lane 5, lung; lane 6, liver; lane 7, skeletal muscle; lane 8, kidney; lane 9, pancreas. Both blots were incubated with a β-actin probe as a control (lower panel). Solid arrows indicate mRNA molecules of the predicted sizes, 1538 and 2247 bp, in retina.

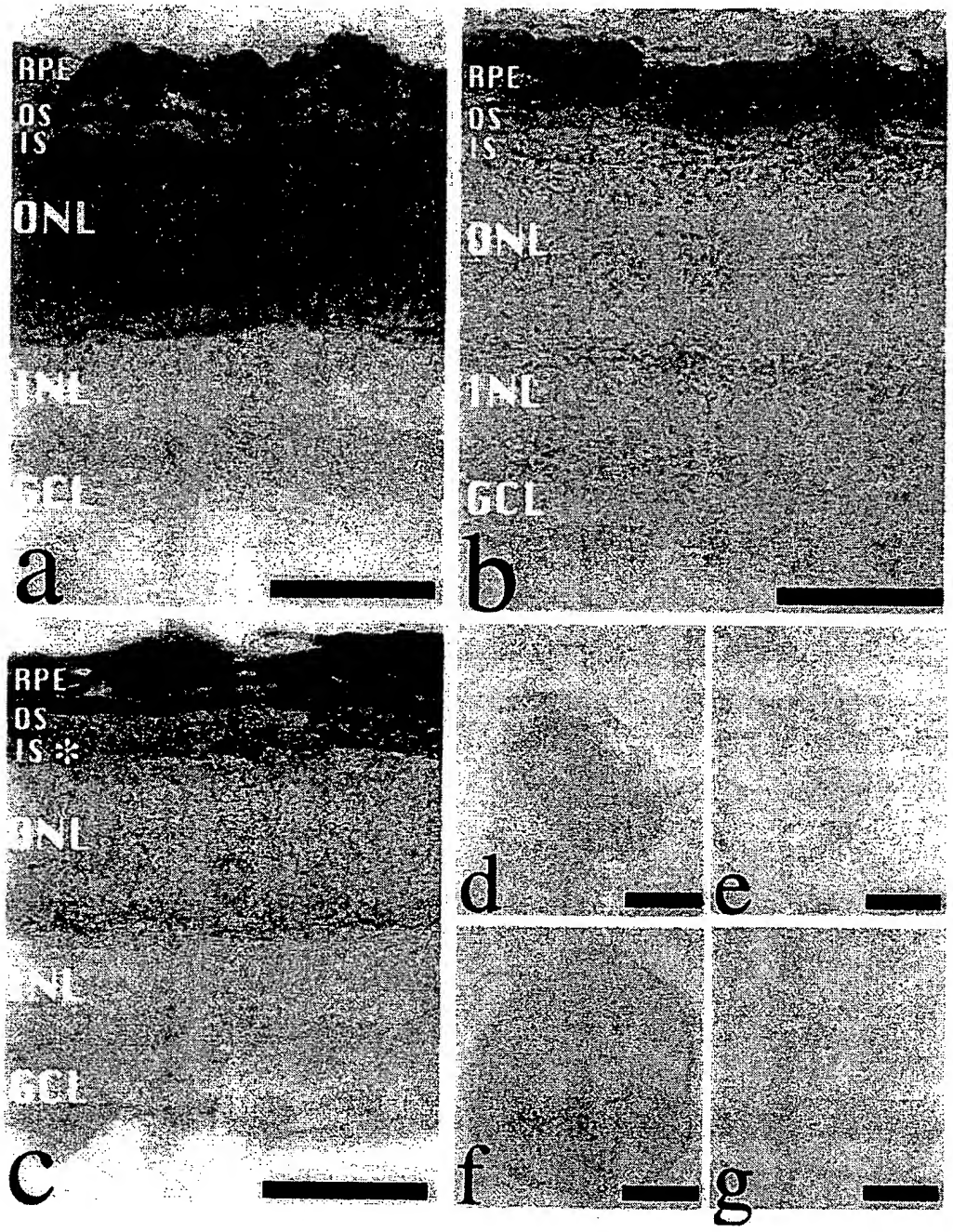

Fig. 4 Retina and pineal expression of *Aipl1*. ***a.*** Digoxygenin *in situ* hybridization of Aipl1 in adult mouse retina, with expression throughout the outer nuclear layer and photoreceptor inner segments. Color reaction time is 4 days. ***b.*** Sense control of "a" with same reaction time. A slight background signal is observed across photoreceptor outer segments. ***c.*** Short (16 hour) color reaction of *Aipl1* in adult mouse retina, showing high level of mRNA in photoreceptor inner segments. ***d.*** Expression of *Aipl1* in adult mouse pineal. Color reaction time is 4 days. ***e.*** Sense control of "d", with same reaction time. ***f.*** Expression of *Aipl1* mRNA in P14 rat pineal. Color reaction time is 4 days. ***g.*** Sense control of "f", with same reaction time. Scale bar for a-c is 30 μm, for d and e is 50 μm, and f and g is 70 μm. RPE-retinal pigment epithelium, OS-outer photoreceptor segment, IS-inner photoreceptor segment, ONL-outer nuclear layer, INL-inner nuclear layer, GCL-ganglion cell layer. Immunolocalization of the AIPL1 protein has not been performed; therefore, site of AIPL1 protein localization is currently unknown.

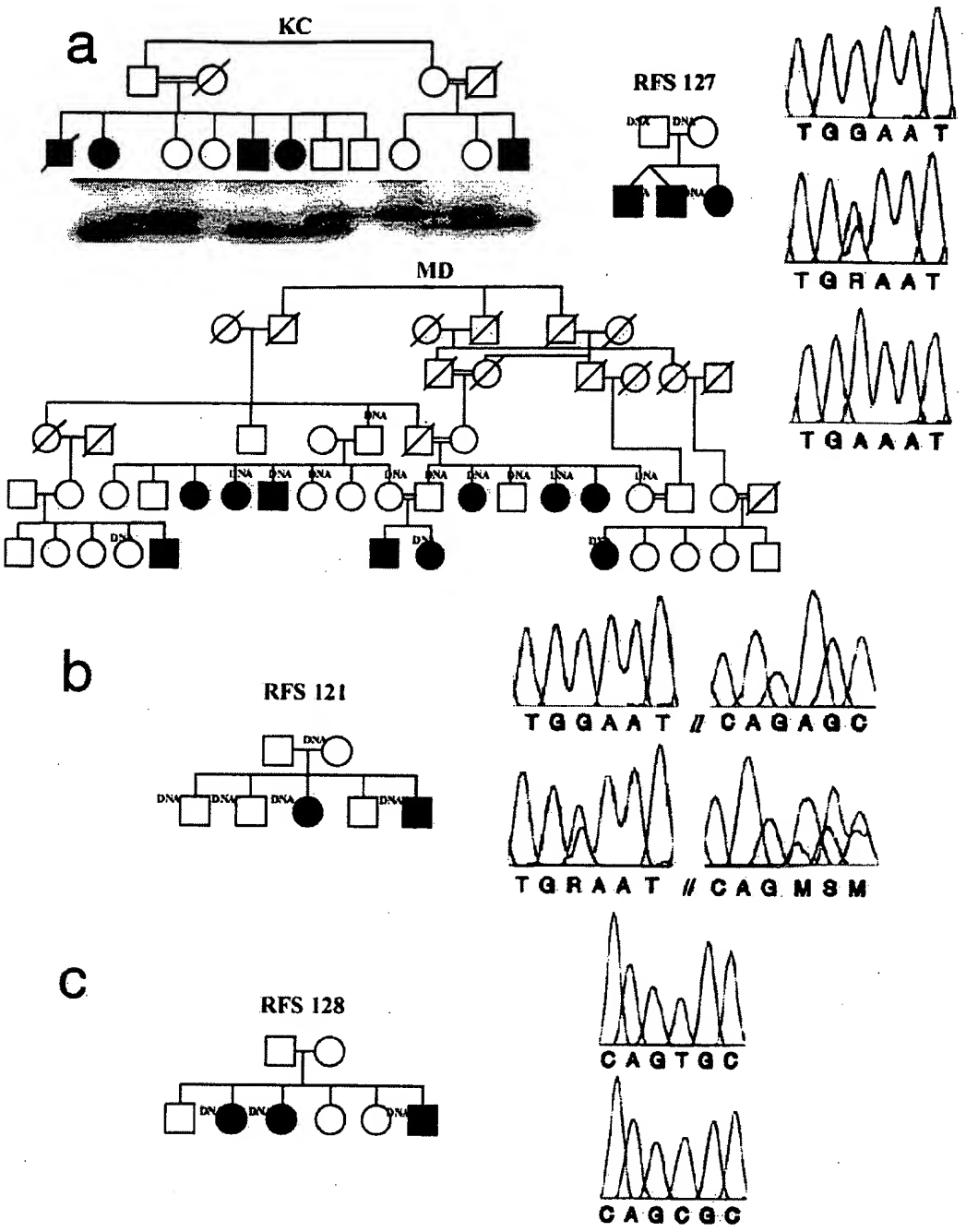

Fig. 5 Pedigrees and mutation screen of AIPL1 in families. ***a.*** The Trp278X mutation is homozygous in three families: KC, MD and RFS127. SSCA of all living individuals of the KC pedigree demonstrate segregation of the mutant allele. Top electropherogram: an unaffected control (TGG/TGG). Middle: heterozygous G/A mutation at codon 278. Bottom: DNA sequence of a homozygous, affected member of MD (TGA/TGA). ***b.*** The RFS121 affected individuals are compound heterozygotes for the Trp278X and Ala336Δ2 bp mutations. Top electropherogram: unaffected control, bottom: heterozygous G/A mutation at codon 278 (left) and heterozygous 2 bp deletion beginning in codon 336 (right) in an affected individual of RFS121. ***c.*** The Cys239Arg mutation found in family RFS128. Top electopherogram: unaffected control (TGC/TGC), bottom: DNA sequence of a homozygous, affected individual (CGC/CGC).

Fig. 6 Fundus photograph of affected LCA patient (eleven years of age), displaying typical symptoms of Leber congenital amaurosis; widespread retinal pigment epithelium changes with pigment clumping, attenuated retinal vessels, pale optic disk, and macular atrophy are evident. Members of the KC family also display keratoconus; because *AIPL1* is not expressed in the cornea, it is possible that this symptom is secondary to LCA in this family, due to eye rubbing, etc.

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